

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 August 2001 (09.08.2001)

PCT

(10) International Publication Number
WO 01/56580 A1

(51) International Patent Classification⁷: **A61K 31/70**

(21) International Application Number: PCT/US01/03064

(22) International Filing Date: 31 January 2001 (31.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/178,950 1 February 2000 (01.02.2000) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/178,950 (CIP)
Filed on 1 February 2000 (01.02.2000)

(71) Applicant (for all designated States except US): **RUTGERS, THE STATE UNIVERSITY** [US/US]; Old Queens Building, Somerset and George Streets, New Brunswick, NJ 08901 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSEN, Robert, T.** [US/US]; 347 Harrier Drive, Monroe Township, NJ 08831 (US). **HO, Chi-Tang** [US/US]; 32 Jernee Drive, East Brunswick, NJ 08816 (US). **DIPAOLA, Robert, S.** [US/US]; 21 Quail Run, Long Valley, NJ 07853 (US).

RAFI, Mohammed [US/US]; 150-A Cedar Lane, Highland Park, NJ 08904 (US). **GHAJ, Geetha** [US/US]; 250 Gallinson Drive, Murray Hill, NJ 07974 (US). **VASTANO, Bret, C.** [US/US]; 8 Easy Street, Lonaka Harbor, NJ 08734 (US).

(74) Agents: **LICATA, Jane, Massey et al.**; Licata & Tyrrell P.C., 66 E. Main Street, Marlton, NJ 08053 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/56580 A1

(54) Title: COMPOSITIONS OF *POLYGONUM ODORATUM* FOR PREVENTION AND TREATMENT OF DISEASE

(57) Abstract: Compositions and methods for prevention and treatment of cancer are provided which comprise extracts of *Polygonum odoratum* or compounds isolated therefrom.

- 1 -

COMPOSITIONS OF *POLYGONUM ODORATUM* FOR PREVENTION
AND TREATMENT OF DISEASE

Background of the Invention

The roots of the plant *Polygonum odoratum* have been used
5 in traditional Chinese medicine for a variety of therapeutic
purposes (Lin, H.W. et al. 1994. *Yao Hsueh Hsueh Pao*. 29:215-
222). It has been used as a crude medicinal agent in the
treatment of analeptic (Tomoda, M. et al. 1971. *Chem.*
Pharmaceut. Bull. 19:2173-2177) and as a nutritious tonic in
10 Asia (Sugiyama, M. et al. 1984. *Chem. Pharmaceut. Bull.*
32:1365-1372). It is referred to as Yu Zhu in China, although
it also grows in Thailand and Vietnam where it is known as Pak
pai or Vietnamese mint. It also grows as a weed in the
southern United States.

15 The methanol extract of the roots of *Polygonum odoratum*
has been shown to suppress 96% of the mutagenicity of Trp-P-1
(Japan International Research Center for Agricultural
Sciences. 1998. 17:1-3). Alcohol extracts have also served
as an immunopotentiator in mice with burn injury (Xiao, J. et
20 al. 1990. *Chung Kuo Chung Yau Tsa Chig.* 15:557-559).
Compounds that have been previously identified in *P. odoratum*
include steroidal saponins (Lin, H.W. et al. 1994. *Yao Hsueh*
Hsueh Pao. 29:215-222), other steroidal compounds (Sugiyama,
M. et al. 1984. *Chem. Pharmaceut. Bull.* 32:1365-1372),
25 cuereitol (Lazer et al. 1971 *Farmacia* 19:31), azetidine 2-
carboxylic acid (Virtanin et al. 1955. *Acta. Chem. Scand.*
9, 551-554; Fowden, L. 1955. *Nature* 176, 347-348), mucous
polysaccharides (Tomoda, M. et al. 1971. *Chem. Pharmaceut.*
Bull. 19:2173-2177), vitamin A, mucilage (Gaal, B. 1927.
30 *Ungar. Pharm. Ges.* 3, 133), and diosgenin (Okanishi et al.
1975. *Chem. Pharm. Bull.* 23, 575-579).

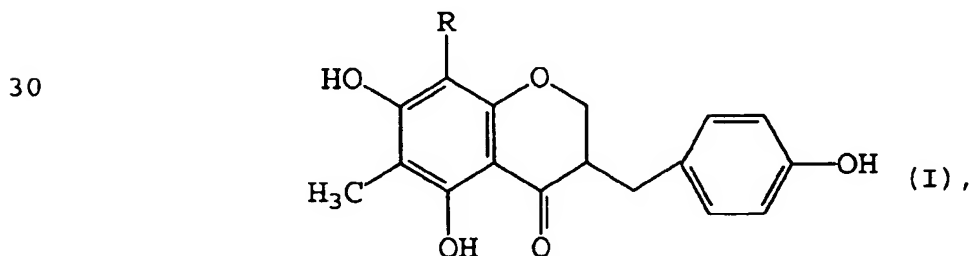
- 2 -

Some natural plant extracts have been shown to have activity as chemopreventive agents. An example, taxol, acts by inducing Bcl-2 phosphorylation in cancer cells which leads to programmed cell death (Halдар, S. et al. 1996. *Cancer Res.* 56:1253-1255). The Bcl-2 protein is a member of a family of cytoplasmic proteins which regulates cell death. Bcl-2 has been shown to promote cell survival by inhibiting the process of cell death known as apoptosis. Where Bcl-2 acts to inhibit apoptosis, Bax, another cytoplasmic protein, counteracts this protective effect; Bcl-2 is also thought to protect cells from apoptosis by dimerizing with Bax (Hunter, J.J. et al. 1996. *J. Biol. Chem.* 271:8521-8524). The phosphorylation of Bcl-2 interferes with the dimerization to Bax resulting in an increase in Bax homodimers and subsequent apoptosis (Halдар, S. et al. 1995. *Proc. Natl. Acad. Sci. USA* 92:4507-4511; Halдар, S. et al. 1996. *Cancer Res.* 56:1253-1255).

It has now been found that extracts of *Polygonum odoratum* have activity to phosphorylate Bcl-2 and induce apoptosis in cancer cells. In addition, two dihydrobenzofuranones which promote Bcl-phosphorylation in breast and prostate cancer cell lines have been isolated from the extract and their structures have been identified.

Summary of the Invention

An object of the present invention is to provide compositions that induce apoptosis in cells which comprise an extract of *Polygonum odoratum* or compounds isolated therefrom. In a preferred embodiment compositions of the present invention comprise a structure of Formula (I):



- 3 -

wherein R is CH₃ or OCH₃.

Another object of the present invention is to provide a method of inducing apoptosis in cells comprising contacting
35 cells with a composition containing an extract of *Polygonum odoratum* or compounds isolated therefrom.

Also provided are methods for preventing and treating cancer which comprise administering an effective amount of a *Polygonum odoratum* extract or compounds isolated therefrom.

40 Detailed Description of the Invention

An extract of *Polygonum odoratum* has been isolated that has use as a cancer preventive agent due to its ability to induce apoptosis in cancer cells. The extract contains novel chemical entities that have activity similar to the
45 chemotherapeutic agent taxol. The extract can be used as a pharmaceutical for cancer treatment and/or prevention as well as a medical food, or nutraceutical and a dietary supplement.

Roots of *Polygonum odoratum* were dried and then ground into a powder. The powdered roots were then extracted with
50 methanol and concentrated under vacuum using rotary evaporation. The remaining concentrate was then partitioned with acidified ethyl acetate (3% HCl) and dried. The dried extract was then chromatographed on a silica gel column for bio-assay directed fractionation. Elution was performed using
55 a solvent mixture of chloroform/methanol with an increasing amount of methanol (30:1, 20:1, 10:1, 8:1, 7:1, 5:1, 3:1, 1:1, 1:5, 1:15, 1:25, 0:1; each 500 ml). Successive fractions were collected and assayed for biological activity. The biological activity was determined by measuring phosphorylation of Bcl-2
60 protein in cells that were treated with various fractions. MCF-7 breast tumor cells were obtained from the ATCC and used. After treatment with a test fraction, cells were lysed and equivalent amounts of proteins were electrophoresed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis
65 (SDS-PAGE) and transferred to nitrocellulose. Bcl-2 protein

- 4 -

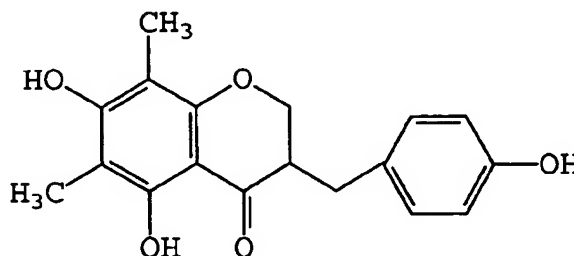
levels were detected using a monoclonal Bcl-2 primary antibody and a secondary goat anti-mouse horseradish peroxidase-conjugated antibody followed by enhanced chemiluminescence detection.

5 An active fraction was yielded upon elution with 30:1 chloroform:methanol mixture. The resulting fraction was re-chromatographed on a second silica gel column and eluted with 20:80:0 hexane:chloroform:methanol to yield an active fraction. This fraction was evaporated to dryness under
10 nitrogen at room temperature. The sample was then reconstituted in ethanol and analyzed by reverse phase HPLC. Two peaks were identified in the chromatogram.

The hexane:chloroform:methanol (20:80:0) fraction was also analyzed by APCI LC-MS in the negative ion mode. It was
15 found that peak 1 had a molecular weight of 330, while peak 2 had a molecular weight of 314.

For determination of structure, isolation and purification of the 20:80:0 hexane:chloroform:methanol fraction was performed for NMR studies. The final
20 purification of the dihydrobenzofuranones was performed using reverse phase, semi-preparative HPLC. Peak 1 was determined to be 2,3-dihydro-3-[(4-hydroxyphenol)methyl]-5,7-dihydroxy-6-methyl-8-methoxy-4H-1-benzopyran-4-one and peak 2 was determined to be 2,3-dihydro-3-[(4-hydroxyphenol)methyl]-5,7-
25 dihydroxy-6,8-dimethyl-4H-1-benzopyran-4-one. The concentration of both dihydrobenzopyranones in the powdered roots of *Polygonum odoratum* was approximately 30 µg/g. The structures of each of the isolated compounds are depicted in Formula (II) and (III), respectively.

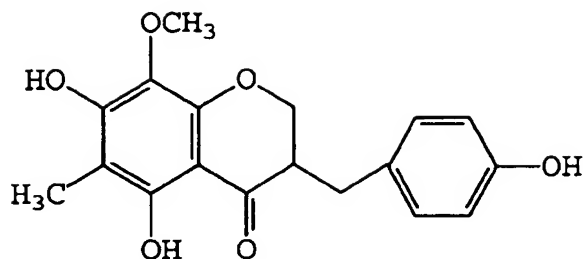
30 (II)



- 5 -

and

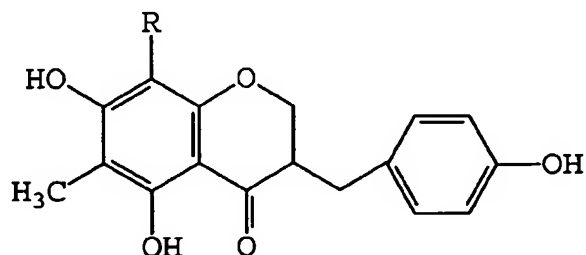
(III)



5 Accordingly, the present invention relates to compositions comprising crude extracts of *Polygonum odoratum* that can be used as a dietary supplement for cancer prevention. The present invention also relates to compositions comprising a purified compound of Formula (I)

10

(I)



wherein R is OCH₃ or CH₃.

Compounds of Formula (I) can be purified from the crude extract and used as pharmaceuticals or dietary supplements for prevention and treatment of cancer. Alternatively, these new
 15 compounds can be prepared synthetically using methods well known to those skilled in the art. Further, one of skill in the art can now develop new compounds with similar structure and activity to that of Formula (I), (II) or (III) for use in the compositions of the present invention based on routine
 20 methods for testing of potential clinical compounds.

The activity of the newly identified compounds shown in Formulas II and III of the present invention was determined in a series of *in vitro* studies in MCF-7 breast cancer cells.

- 6 -

First the effects of the compounds of Formula II and III on cell growth was examined in a cell cycle assay. In this assay, the number of cells in the G_0 - G_1 , S and G_2 -M phases were first determined without treatment with any test compounds (control data). These control data showed that 72.19% of cells were in the G_0 - G_1 phase, 10.12% were in the G_2 -M phase while 17.69% were in the S phase. Then, the breast cancer cells were treated with a positive control compound, paclitaxel (5 μ M concentration), which is the standard drug of choice in the clinics for treatment of breast cancer. Treatment with paclitaxel resulted in 54.93% of cells in the G_2 -M phase, indicating that paclitaxel arrested 54.93% of cells at that phase, thus blocking cell cycle progression and preventing further cell cycle progression and growth of the cells. Next, MCF-7 cells were treated with the compound of Formula II at a concentration of 50 μ M. At this dose, the test compound arrested 15.09% of cells at the G_2 -M phase, showing that the compound had some effect on cell growth. However, treatment of the cells with a 25 μ M concentration of the compound of Formula III resulted in 42.22% of cells arrested at the G_2 -M phase. These data show that the compound of Formula III had effects similar to the standard drug paclitaxel on cell cycle progression and growth.

Cell viability was also examined in the presence of the compounds of Formulas II and III. These studies were performed by plating breast cancer cells in the presence and absence of the compounds of Formulas II and III. The cells were contacted with increasing concentrations of the two test compounds and then allowed to grow for 15 to 21 days. The colonies formed were stained with methylene blue and the number of colonies was counted. The results showed that the compound of Formula II produced a dose-response inhibition of cell growth. The number of colonies counted decreased from more than 1000 in the control plates to approximately 600 colonies at a 50 μ M concentration of the Formula II compound,

- 7 -

and less than 50 colonies at a 100 μM concentration of the compound of Formula II. A dose-response effect also was seen in the presence of the compound of Formula III, with the number of colonies decreasing from more than 1000 in control conditions, to less than 600 colonies in the presence of 10 μM of the compound of Formula III, less than 300 colonies in the presence of 20 μM of the compound of Formula III, less than 50 colonies in the presence of 50 μM of the compound of Formula III, and less than 25 colonies in the presence of 100 μM of the compound of Formula III. These data showed that the compound of Formula III was a potent inhibitor of cell growth and viability.

The ability of the test compounds of Formulas II and III to induce Bcl-2 phosphorylation was examined by Western blot analysis. The presence of Bcl-2 and phosphorylated Bcl-2 proteins was detected using a monoclonal Bcl-2 primary antibody and a secondary goat anti-mouse HRP-conjugated antibody. The results showed that control breast cancer MCF-7 cells treated with vehicle (alcohol) had no detectable phosphorylated Bcl-2. Cells treated with 1 μM paclitaxel showed significant levels of phosphorylated Bcl-2 protein. Breast cancer cells treated with 10, 20, 40 or 80 μM of the compound of Formula III demonstrated a dose-response increase in levels of phosphorylated Bcl-2 protein. In contrast, cells treated with 10, 20, 40, 80 or 100 μM of the compound of Formula II showed no significant levels of phosphorylated Bcl-2 protein. These data demonstrate the ability of the compound of Formula III to induce Bcl-2 phosphorylation in cancer cells, results consistent with the effects of a known chemotherapeutic agent, paclitaxel, in cancer cells. The ability to induce Bcl-2 phosphorylation is known to be a precursor event to programmed cell death.

These data demonstrate the pharmacological activity of the compositions of the present invention as therapeutics in the prevention and treatment of cancer. Compositions of the

- 8 -

present invention can be administered either orally as a dietary supplement or by other methods routine in the art including, but not be limited to, intravenous, intraperitoneal, subcutaneous, or intramuscular injection.

5 In a preferred embodiment, compositions of the present invention are formulated in a pharmaceutically acceptable vehicle. Such vehicles include, but are not limited to, aqueous solutions such as saline. The pharmaceutically acceptable vehicle is selected based on the solubility
10 characteristics of the compound to be administered and the route of administration. Such selection is routine to one of skill in the art.

Compositions of the present invention can be administered to animals, including humans, for preventing or
15 treating cancer. The animals to be treated can be administered an effective amount of the compounds of the present invention for prevention or treatment of cancer where an effective amount is defined as an amount that induces apoptosis in cancer cells.

20 The following non-limiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Fractionation and Purification

¹H NMR and ¹³C NMR spectra were obtained on a VXR-200
25 instrument and MS analysis was performed on a Micromass Platform II system (Micromass Co., MA) equipped with a Digital DECPc XL5650 computer for analysis of data. Mass spectra were obtained using atmospheric pressure chemical ionization (APCI) in the negative-ion mode. The ion source temperature was set
30 at 150 C and the probe temperature was set at 450 C. The sample cone voltage was 10 V and the corona discharge was 3.2 kV. The HPLC analysis was performed on a Varian Vista 5500 liquid chromatograph pump coupled to a Varian 9065 Polychrom diode array detector. Fractionation of purified compounds

- 9 -

was obtained on a Waters 600E HPLC pump coupled to a Milton Roy Spectro Monitor 3100 variable wavelength detector. Selecto Scientific silica gel (100-200 mesh particle size) was used for column chromatography. All fractions were screened
5 on Whatman silica gel thin-layer chromatography plates (250 μ m thickness, 60 A silica gel medium) with compounds revealed under fluorescent light.

Example 2: Apoptosis by Annexin V Assay

Apoptosis was assessed by the Annexin V fluorescence
10 isothiocyanate (FITC) method. Apoptosis detects changes in the position of phosphatidylserine (PS) in the cell membrane. In nonapoptotic cells, most PS molecules are localized at the inner layer of the plasma membrane. Once apoptosis is induced, PS redistributes to the outer layer of the membrane
15 and becomes exposed to the extracellular environment. The exposed PS can be easily detected with annexin V, a 35.8 kDa protein with a strong affinity for PS. Annexin V is conjugated as a fusion protein with enhanced green fluorescent protein (EGFP). This assay is nonenzymatic and can be used with
20 fluorescence microscopy. Early and late stage apoptotic cells were seen as green fluorescence while late stage necrotic cells were identified by a yellow-red intracellular staining appearance.

Example 3: Cell Viability Assay

25 Cell viability was assessed by the microculture tetrazolium/formazan assay (MTT; Scudiero, D.A. et al. 1988. Cancer Res. 48:4827-4833). Absorbance was measured at 550 nm. Cell viability was expressed as the percentage of drug treated cells relative to that of the controls. The IC_{50} was defined
30 as the concentration of drug that produced a 50% decrease in cell viability relative to controls.

- 10 -

Example 4: Western Blot Analysis

Analysis of Bcl-2 protein was performed by immunoblots. Cells were initially treated with various fractions and pure compounds. Cells were then lysed in ice cold radio-immune precipitation buffer with inhibitors. Equivalent amounts of proteins were electrophoresed by 12% dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Bcl-2 and phosphorylated Bcl-2 proteins were detected using a monoclonal Bcl-2 primary antibody and a secondary goat anti-mouse horseradish peroxidase conjugated antibody followed by enhanced chemiluminescence detection (Haldar, S. et al. 1994. *Arch. Biochem. Biophys.* 315:483-488).

Example 5: Cell Cycle analysis

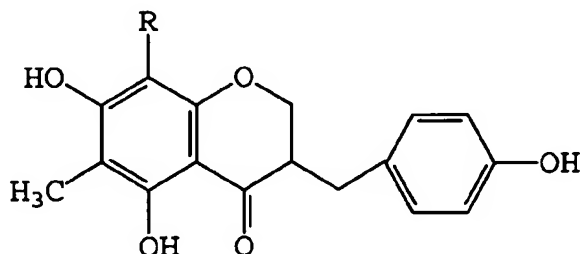
Cells were treated for 12 hours, incubated with 10 μ M BrdU for 45 minutes at 37°C. Cells were then washed with ice-cold PBS, resuspended in 200 μ l PBS and fixed with cold 70% ethanol. The cells were resuspended, incubated for 30 minutes in 2 N hydrochloric acid /0.5% Triton X-100 in PBS, and neutralized by rinsing once in 0.1 M sodium tetraborate (pH 8.5). Fluorescein isothiocyanate (FITC)-conjugated anti-BrdU antibody (Becton-Dickinson) were added (10 μ g per sample) in 50 μ l of 0.5% Tween 20/1% BSA in PBS and incubated for 30 minutes. The cells were washed and resuspended in 1 ml of PBS containing 5 μ g/ml propidium iodide. Fluorescence intensity was determined by quantitative flow cytometry and profiles were generated on Becton Dickinson FACScan. A minimum of 10,000 cells were analyzed using Modifit LT (Verily Software House, Inc.).

- 11 -

What is claimed is:

1. A composition that induces apoptosis in cells comprising an extract of *Polygonum odoratum* or compounds isolated therefrom.

5 2. The composition of claim 1 wherein the isolated compound comprises Formula (I):



wherein R is CH₃ or OCH₃.

3. The composition of claim 1 further comprising a pharmaceutically acceptable vehicle.

10 4. A method for inducing apoptosis in cells comprising contacting cells with the composition of claim 1.

5. A method for preventing or treating cancer in an animal comprising administering to an animal an
15 effective amount of the composition of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/03064

A. CLASSIFICATION OF SUBJECT MATTER												
IPC(7) : A61K 31/70 US CL : 514/44												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/44												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched 514/44												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Word Searched West; polygonum same cancer and apoptosis; Rhizoma Polygonati Odorati; Searched STN: file caplus; d que nos 16; file uspatfull; d que nos 17; d ibib abs hitstr Search Terms STN: the structure of claim 2												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X	PA0-LIN et al., Benzoquinones A Homoisoflavnone and Other Constituents from Polygonatum Alte-Lobatum, Phytochemistry 1997, Vol. 44, No. 7, pages 1369-1373, especially page 1371.	1-3										
A,P	US 6,197,754 B1 (HUNG et al) 20 March 2001 (20.03.01), column 18, lines 34-36	4										
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.												
* Special categories of cited documents: <table border="0"> <tr> <td>- "A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>- "E" earlier application or patent published on or after the international filing date</td> <td>- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>- "O" document referring to an oral disclosure, use, exhibition or other means</td> <td>- "&" document member of the same patent family</td> </tr> <tr> <td>- "P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			- "A" document defining the general state of the art which is not considered to be of particular relevance	- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	- "E" earlier application or patent published on or after the international filing date	- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	- "O" document referring to an oral disclosure, use, exhibition or other means	- "&" document member of the same patent family	- "P" document published prior to the international filing date but later than the priority date claimed	
- "A" document defining the general state of the art which is not considered to be of particular relevance	- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
- "E" earlier application or patent published on or after the international filing date	- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
- "O" document referring to an oral disclosure, use, exhibition or other means	- "&" document member of the same patent family											
- "P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search		Date of mailing of the international search report 20 APR 2001										
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-7939		Authorized officer <i>Randall Winston</i> Telephone No. 703-305-0404										

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Inte _ onal application No.

PCT/US01/03064

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See Attachment

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-4

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/03064

Inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-4, drawn to a composition and a method of inducing apoptosis cells by using the composition.

Group II, claim 5, drawn to a method of preventing and treating cancer in animals by administering to animals group's I composition.

The inventions listed as Group I and Group II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of Group I is drawn to a composition and a method of inducing apoptosis cells by using the composition. Group II is drawn to a method of preventing and treating cancer in animals by administering to animals group I's composition. Thus, Group I and Group II are different because although the same composition would be utilized in both inventions, the composition would perform different functions in each separate invention to achieve different purposes.